Cat. # 6141

For Research Use

TaKaRa

Takara IVTpro™ mRNA Synthesis System

Product Manual

v202207Da

Table of Contents

I. Description

Takara IVTpro mRNA Synthesis System comprises of two kits, which is designed to construct plasmid template of in vitro transcription (IVT) using TriLink BioTechnologies cap analogs and to prepare high-yield mRNA of target gene by IVT from IVT template.

(1) Cloning Kit for mRNA Template for easy construction of the template plasmid containing a coding sequence (CDS) of a desired gene used in *in vitro* transcription (IVT) using TriLink BioTechnologies cap analogs [CleanCap Reagent AG or CleanCap Reagent AG (3′ OMe)].
(2) Takara IVTpro T7 mRNA Synthesis Kit for synthesizing high-quality, high-yield CleanCap capped mRNA through *in vitro* transcription (IVT) using the plasmid template constructed with (1).

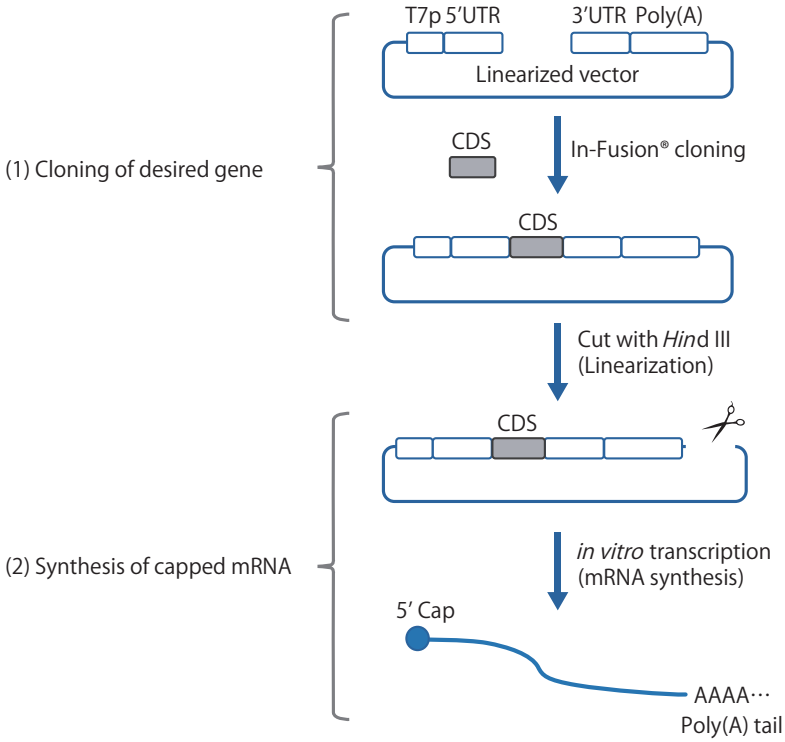

Fig. 1. Overview of experimental flow of this product.

(1) Cloning Kit for mRNA Template (Cat. #6143)

The kit is designed for easy construction of the template plasmid used in *in vitro* transcription. The Linearized Template Vector in the kit contains a T7 promoter, transcription start sequence (AGG), 5′-UTR (untranslated region), 3′-UTR, and a 105-base Poly(A) sequence, which make it possible to construct an IVT template plasmid for mRNA synthesis for expression in human, murine, or other mammalian cells using CleanCap Reagent AG by In-Fusion cloning. CleanCap Reagent AG is a co-transcriptional capping reagent for the capping of mRNA, creating a Cap 1 structure.

(2) Takara IVTpro T7 mRNA Synthesis Kit (Cat. #6144)

This kit is designed for synthesizing mRNA through an *in vitro* transcription (IVT) using double-stranded DNA (containing the T7 promoter) as a template. It works efficiently with CleanCap Reagent AG to add a cap 1 structure to the 5′ end of the mRNA, which is needed for efficient protein translation in eukaryotes. Moreover, modified NTPs, such as pseudo-UTP, etc., can be used instead of UTP to reduce the innate immune response (Kariko, *et al.*, 2008) of mRNA in the transfected cells without affecting mRNA yield. For details, refer to the instruction manual of Takara IVTpro T7 mRNA Synthesis Kit (Cat. #6144).

II. Components

Cloning Kit for mRNA Template*1 (Cat. #6143) (10 reactions)

LTV Linearized Template Vector (50 ng/μl)	10 μl
FLuc FLuc Control Fragment (100 ng/μl)*2	10 μl
In-Fusion 5X In-Fusion Snap Assembly Master Mix*3	20 μl

Takara IVTpro T7 mRNA Synthesis Kit*1 (Cat. #6144) (20 reactions, 20 μl volume)

TB 10X Transcription Buffer	40 μl
ATP 10X ATP	40 μl
CTP 10X CTP	40 μl
GTP 10X GTP	40 μl
UTP 10X UTP	40 μl
EM 10X Enzyme Mix	40 μl
H_2O Nuclease-Free Water	1 ml x 3
DNase I DNase I	80 μl
LiCl Lithium Chloride Precipitation Solution	600 μl
PCT Positive Control Template (FLuc) (0.5 μg/μl)*4	10 μl

*1 Each kit is also sold separately.

*2 Control DNA fragment for In-Fusion cloning, which contains *Photinus pyralis* luciferase CDS, optimized for use in human cells.

*3 Same as that in In-Fusion Snap Assembly Master Mix (Cat. #638943/638944/638947 - 638949)

*4 Linearized plasmid template containing T7 promoter + 5′UTR + FLuc-CDS + 3′UTR + Poly(A)

III. Storage -20℃

IV. Materials Required but not Provided

A. Reagents

- Competent cells
  - Stellar™ Competent Cells (Cat. #636763), etc.
- SOC medium
- Luria-Bertani (LB) medium
- LB /kanamycin (50 μg/ml) plate
- *Hin*d III (Cat. #1060A/B)
- Cap analog
  - CleanCap Reagent AG (TriLink BioTechnologies, No. N-7113-1/5/10)
  - CleanCap Reagent AG (3′ OMe) (TriLink BioTechnologies, No. N-7413-1/5/10)
- Modified NTP
  - N^1-Methylpseudouridine-5′-Triphosphate
  - Pseudouridine-5′-Triphosphate
  - 5-Methoxyuridine-5′-Triphosphate
  - 5-Methylcytidine-5′-Triphosphate, etc.
- Ethanol
- 3M sodium acetate (pH 5.2)
- TE buffer (containing 0.1 mM EDTA)

B. Equipment

- Reaction tubes
- Micropipettes, and tips
- Constant temperature bath or thermal cycler
- Refrigerated micro centrifuge
- Spectrophotometer
  - NanoDrop (Thermo Fisher Scientific), etc.

V. General Considerations

RNase contamination of the double-stranded DNA template, reagents, tubes, micropipette tips, or other materials used in the reaction can significantly decrease or digest RNA obtained with the kit. Use dedicated tubes and micropipette tips in the reaction and wear new disposable gloves to prevent RNase contamination.

VI. Cloning of Desired Gene Using Cloning Kit for mRNA Template

VI-1. Product overview

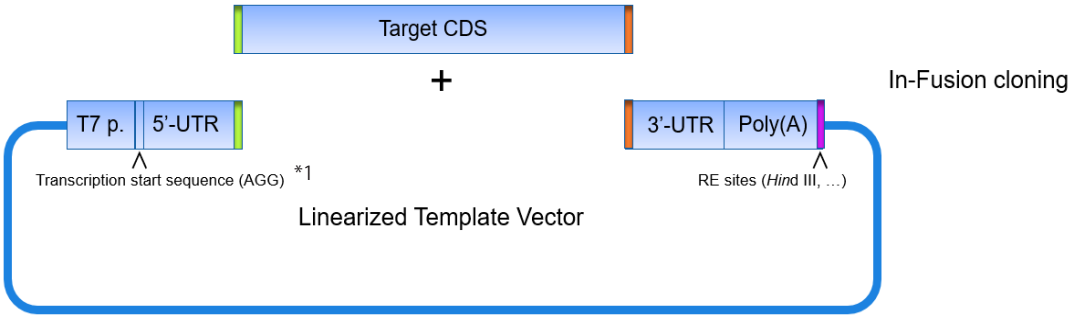

Fig. 2A. IVT template Plasmid construction by In-Fusion cloning*2.

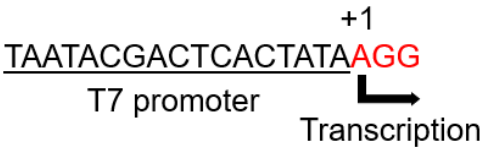

Fig. 2B. Transcription start sequence (AGG) of IVT template when CleanCap Reagent AG is used.

*1 The "transcription start sequence" (AGG) is needed to efficiently prepare capped RNA with CleanCap Reagent AG.

*2 For more information on In-Fusion Snap Assembly Master Mix (Cat. #638943/638944/638947 to 638949), please see our website: https://www.takarabio.com

VI-2. Preparation of IVT Template Plasmid

A) Coding sequence (CDS) design of desired gene

1. Obtain the CDS sequence for the desired gene.
   - In order to express a certain gene, you need its CDS (DNA sequence from the start codon to the stop codon). Even to express a part of a gene, the start and stop codons must be added (See "VI-2-B, PCR amplification of the desired CDS fragment").
   - Even though the vector also contains a stop codon, prepare a CDS containing the stop codon to complete translation of the desired protein reliably.

2. Optimize the CDS codons for the cell types into which the mRNA will be transfected.
   - Use online tools or commercially available software.
   - When RNA is synthesized by *in vitro* transcription, a pseudo-UTP is often used instead of UTP to reduce the immunogenicity in mammalian cells (Kariko *et al.*, 2008). However, reduction of uridine (U) usage in the sequence is also important for immunogenicity reduction (Vidyanathan *et al*., 2018 and Xia 2021). Design the CDS considering both specific codon optimization and the frequency of uridines (U).

3. Confirm that the restriction site (*Hin*d III; recommended) used for linearizing the IVT template plasmid is not present in the CDS of the desired gene. If the restriction site *Hin*d III is present in the CDS, change the DNA sequence by changing the codon in the restriction sequence while keeping the amino acid sequence the same (e.g., switch from serine codon "UCU" to "UCC).

   Note: We strongly recommend using *Hin*d III to linearize the plasmid containing the CDS.

   - To synthesize IVT transcripts of uniform length, linearize the template plasmid with a restriction enzyme. Cutting the template plasmid using restriction enzymes that produce a 3' overhang may produce undesired RNA transcripts that correspond to the antisense strand or to the vector DNA (Schenborn *et al*., 1985). Therefore, we recommend plasmid linealization with a restriction enzyme that will produce a 5' overhang or a blunt end. Moreover, addition of extra bases after the Poly(A) sequence in the mRNA may cause a decrease in the translation efficiency in some cases. Whenever possible, use a restriction enzyme site that will not leave any extra bases.

4. Prepare the CDS of the desired gene, using DNA synthesis and cDNA cloning, etc.

B) PCR amplification of the desired CDS fragment

1. As shown below, the 15-base sequence (red) for In-Fusion cloning is added to the 5′ end of the Forward and Reverse primers that can amplify the CDS of the desired gene.
   - Start codon (blue) and stop codon (green: complementary strand) of the CDS
   - When the CDS containing the In-Fusion sequence is prepared by DNA synthesis, proceed to "V-2-C) In-Fusion cloning".

   Example: FLuc

   Forward primer:
   5′-AGAGAACCCGCCACCATGGAGGACGCCAAGAACATCAA-3′

   Reverse primer:
   5′-CGAGGCTCCAGCTCATCACACGGCGATCTTGCCGC-3′

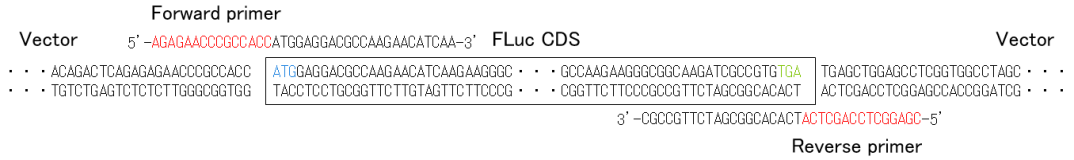

Fig. 3. PCR primer for FLuc CDS fragment

2. Perform PCR amplification of the CDS with the primers above. The resulting amplicon has a region of 15-base overlap with the ends of the ⓘ Linearized Template Vector.
   - For PCR amplification, we recommend using PrimeSTAR® Max DNA Polymerase (Cat. #R045A/B), which has the highest level of accuracy, or TaKaRa Ex Premier™ DNA Polymerase (Cat. #RR370S/A/B, RR371S/A/B), which has a high PCR success rate in addition to high accuracy.

3. Perform agarose gel electrophoresis with 5 μl of the PCR solution and confirm the amplified PCR product and its quantity.
   - About 50 to 100 ng/μl of the purified PCR product is needed for In-Fusion cloning (VI-2-C). Confirm that you have a sufficient amount.

4. Purify the PCR product with spin column purification kit (NucleoSpin Gel and PCR Clean-up; Cat. #740609.10/.50/.250).
   - If not using the DNA immediately after purification, store it at -20℃.
   - If there are multiple amplification products, purify the DNA from the desired PCR band, optimize the PCR conditions, or redesign the primer.

C) In-Fusion Cloning

1. Thaw the LTV Linearized Template Vector and CDS fragment from VI-B-4 at room temperature and spin down after mixing briefly.

2. Prepare the reaction solution as follows.

< Per reaction >

Reagent	Volume
LTV Linearized Template Vector (50 ng/μl)	1 μl
CDS fragment with In-Fusion sequence*	100 ng
Nuclease-Free Water	X μl
In-Fusion 5X In-Fusion Snap Assembly Master Mix	2 μl
Total	10 μl

* If using FLuc FLuc Control Fragment, use 1 μl (100 ng).

3. Incubate at 50℃ for 15 minutes.

Note: If not proceeding with transformation immediately, store the reaction solution on ice or at -20℃ for longer storage.

D) Transformation

Follow the protocol below for the transformation of Stellar Competent Cells (Cat. #636763). Use the recommended competent cells or ones with a transformation efficiency of at least 1 x 10^8 cfu/μg.

1. Thaw the competent cells on ice.
2. After light mixing, transfer 50 μl of the competent cells to a fresh tube.
3. Add 2.5 μl of reaction solution prepared in Step C, mix lightly, and let stand on ice for 30 minutes.
4. Heat shock the competent cells at 42℃ for 45 seconds.
5. Cool them on ice for 1 to 2 minutes.
6. Add 450 μl of SOC medium and shake-culture at 37℃ for 1 hour.
7. Spread 50 μl of the culture medium from Step 6, and a 10-fold dilution with SOC medium on LB plates containing kanamycin and culture overnight at 37℃.

E) Expected results

When FLuc FLuc Control Fragment is used, 100 or more colonies will ordinarily be obtained from plating 50 μl of the undiluted medium. Make liquid cultures of the individual colonies and purify the plasmid by the standard method (using NucleoSpin Plasmid, Cat. #740588.10, etc.). Confirm the sequence of the purified plasmids. See the product page for sequence information for the LTV Linearized Template Vector and the FLuc FLuc Control Fragment cloned in Linearized Template Vector.

- The Poly(A) sequence may be truncated depending on the *E. coli* strain or culturing method. We recommend confirming the sequence of the colony-derived plasmid and then preparing several glycerol stocks of the verified *E. coli* clone.

  Use the following sequencing primers to confirm the presence of the Poly(A) sequence.
  Poly(A) Forward primer: 5′-CCTCGGTGGCCTAGCTTCTT-3′
  Poly(A) Reverse primer: 5′-CAGGGCTTCCCAACCTTACC-3′

VII. Preparation of Linearized Plasmid Template for Takara IVTpro mRNA Synthesis Kit

A. Preparation of linearized IVT plasmid template

a) Restriction enzyme treatment

A plasmid template DNA concentration of 0.5 to 1.0 μg/μl is needed for IVT. Refer to the following example for restriction enzyme treatment. Account for recovery loss following ethanol precipitation or column purification after restriction enzyme treatment.

Example

< Per reaction >

Reagent	Volume
Template Plasmid	50 μg
10X M Buffer	20 μl
Nuclease-Free Water	X μl
*Hin*d III (15 U/μl)	10 μl
Total	200 μl

Incubate at 37℃ for 3 hours.

Note: If the restriction digestion is incomplete and some uncut circular template plasmid remains, some of RNA synthesized by IVT will be at a size larger than desired. Confirm complete linearization of the plasmid by agarose gel electrophoresis using 5 μl of the reaction solution.

b) Ethanol precipitation

1. Add 1/10 amount of 3M sodium acetate (pH 5.2) and twice the amount of ethanol to the restriction digestion solution.
2. Mix well and cool at -20℃ for at least 15 minutes.
3. Centrifuge for 15 minutes at maximum speed at 4℃.
4. Carefully remove the supernatant using a pipette, add 1 ml of 70% ethanol, and centrifuge again under the same conditions.
5. Carefully remove the supernatant and dry the pellet.
6. Dissolve the DNA in H2O Nuclease-Free Water or TE buffer (containing 0.1 mM EDTA) and measure the concentration of the DNA. If necessary, adjust the concentration to between 0.5 to 1.0 μg/μl. Store the IVT plasmid template solution at -20℃ until use.

   Note: Fragmentation of the mRNA synthesized using the linearized plasmid template above may indicate RNase contamination. To prevent RNA degradation, perform phenol-chloroform extraction of the plasmid template after restriction digestion and then purify it by ethanol precipitation.

B. Preparation of the PCR template

When using a PCR product as IVT template, perform PCR amplification of the sequence from T7 promoter to the Poly(A). Prepare IVT template following the same purification method described above.

VIII. IVT Reaction

1. Preparation of reagents
   - Thaw components other than (EM) 10X Enzyme Mix in Takara IVTpro mRNA Synthesis Kit at room temperature. Mix gently, and spin down.
   - Spin down the (EM) 10X Enzyme Mix briefly and keep it on ice until use (do not vortex).

2. Prepare the reaction solution as shown below at room temperature.
   * Be sure to add each component in the **order shown**.
   Calculate the amount of Nuclease-Free Water needed beforehand.

mRNA synthesis reaction using CleanCap Reagent AG or CleanCap Reagent AG (3′ OMe)

< Per reaction >

Reagent	Volume
(H_2O) Nuclease-Free Water	X μl
(TB) 10X Transcription Buffer*1	2 μl
(ATP) 10X ATP*2	2 μl
(CTP) 10X CTP*2	2 μl
(GTP) 10X GTP*2	2 μl
(UTP) 10X UTP*2	2 μl
CleanCap Reagent AG*3	1.6 μl
Template DNA*4	1 μg
(EM) 10X Enzyme Mix	2 μl
Total*5	20 μl

*1 (TB) 10X Transcription Buffer contains spermidine, which forms a complex with nucleic acid and in some cases may precipitate out as an insoluble material. Be sure to add the components in the **order shown**.

*2 **The concentration of each NTP is 100 mM.** When using a modified NTP, replace the corresponding NTP with an equivalent amount.

*3 Use CleanCap Reagent AG or CleanCap Reagent AG (3′ OMe) at a 4:5 molar ratio with NTP (final concentration 8 mM) (See Fig. 4 A/B).

*4 Use a template that has the AGG transcription start sequence. The optimal amount of template differs depending on the size and type of template used, but you can ordinary use a range between 0.5 to 2 μg (See Fig. 5). For (PCT) Positive Control Template (FLuc), use 2 μl (1 μg).

*5 Scale-up as necessary (See Fig. 6A/B).

3. Mix well and incubate at 37℃ for 2 hours.
   - Adjust the reaction time depending on the target length and the desired RNA yield (See Fig. 7 A/B).
   - A white precipitate may form at the end of the reaction. This will likely be magnesium pyrophosphate produced when the pyrophosphate released by the reaction reacts with the magnesium in the solution. This precipitate does not affect subsequent operations, so proceed to the next step, "IX. DNase I Treatment."

IX. DNase I Treatment

After the reaction in VIII-3, add 4 μl of DNase I, mix briefly and incubate at 37℃ for 15 minutes.

X. Purification by LiCl Precipitation

The LiCl precipitation can effectively remove uncaptured NTPs and proteins. However, RNA cannot be efficiently recovered if the RNA size is less than 300 bases or the RNA concentration is less than 0.1 μg/μl. If RNA are a smaller size or concentration less than 0.1 μg/μl, purify the RNA by spin column (NucleoSpin RNA Clean-up, Cat. #740948.10/.50/.250) or ethanol precipitation after phenol-chloroform extraction. RNA purified by these methods can be used in transfection, electroporation, and microinjection experiments (See Fig. 7 A/B).

1. Thaw LiCl Lithium Chloride Precipitation Solution at room temperature before use. Mix well, and if a precipitate is observed, warm the solution at 37℃. If the precipitate persists, use as is. The precipitate does not affect the subsequent steps.
2. Add 30 μl of H_2O Nuclease-Free Water and 30 μl of LiCl Lithium Chloride Precipitation Solution to the IVT reaction solution (about 24 μl) treated with DNase I in Step IX to stop the reaction.
3. Mix well, then cool at -20℃ for at least 30 minutes.
4. Centrifuge at 4℃ for 15 minutes at maximum speed.
5. Carefully remove the supernatant and wash the pellet with 1 ml of 70% ethanol.
6. Centrifuge again at 4℃ for 15 minutes at maximum speed.
7. Carefully remove the supernatant.
8. Air-dry the pellet and dissolve in 100 μl of H_2O Nuclease-Free Water.
   Note: Excessive air drying will make resuspension in H_2O Nuclease-Free Water difficult.
   Note: Depending on the RNA yield, the pellet may take time to dissolve. Let stand at room temperature or 4℃, and mix as needed.
9. After dissolving, measure the RNA concentration with NanoDrop, etc. If not using the RNA sample immediately, store at -20℃.
   Note: Presence of residual unused NTP, cap analogs, or the template DNA will affect the OD measurement. Measure a sample that has been purified by the method described above.
   Note: As needed, confirm the length and purity of the RNA by denaturing agarose/acrylamide gel or Bioanalyzer (Agilent).

XI. Experimental Examples

Example 1-A

< Method >
FLuc mRNA was synthesized in IVT reaction solution with or without CleanCap Reagent AG (3′ OMe).

< Results >
Use of CleanCap had almost no effect on RNA yield.

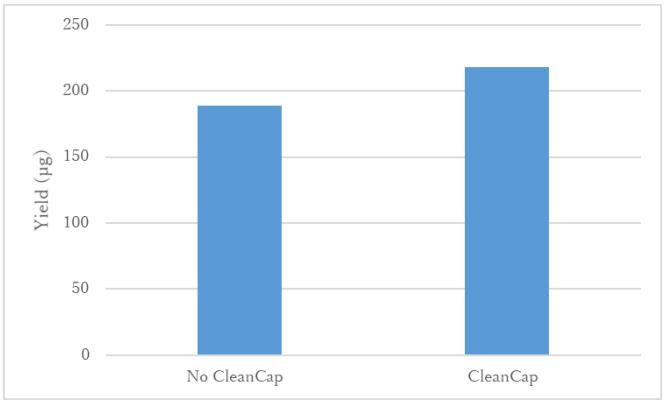

Fig. 4A. Affect of CleanCap Reagent AG (3′ OMe) on RNA yield.

Example 1-B

< Method >
HEK293T cells were transfected with 0.5 µg of the RNA obtained in Example 1-A, using *Trans*IT-mRNA Transfection Kit (Mirus Bio, No. MIR2225).

< Results >
The cells were recovered 24 hours after transfection and FLuc luciferase activity was measured. The FLuc mRNA synthesized in IVT reaction with CleanCap showed activity equivalent to that of the commercially available FLuc mRNA positive control. By contrast, no activity was observed in the FLuc RNA synthesized in the IVT reaction without CleanCap. This shows that addition of a cap is essential for protein expression by the resulting mRNA.

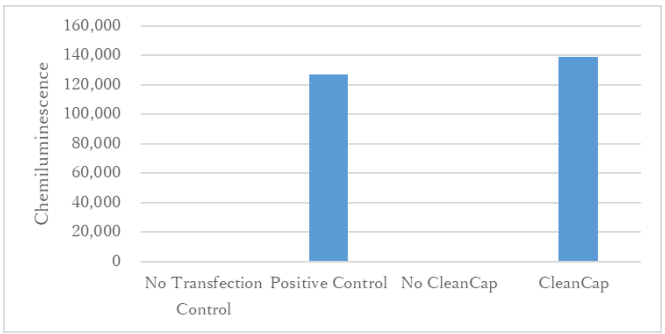

Fig. 4B. Expression of FLuc mRNA synthesized using CleanCap Reagent AG (3′ OMe) in HEK293T cells.

Example 2

< Method >
IVT reaction (20 μl) was performed using various amounts of Positive Control Template (FLuc) with CleanCap Reagent AG (3′ OMe) and N^1-methyl pseudo UTP.

< Results >
The RNA yield peaked when the amount of DNA was 0.5 μg, and no change in yield was observed over 0.5 μg (0.5 to 2 μg).

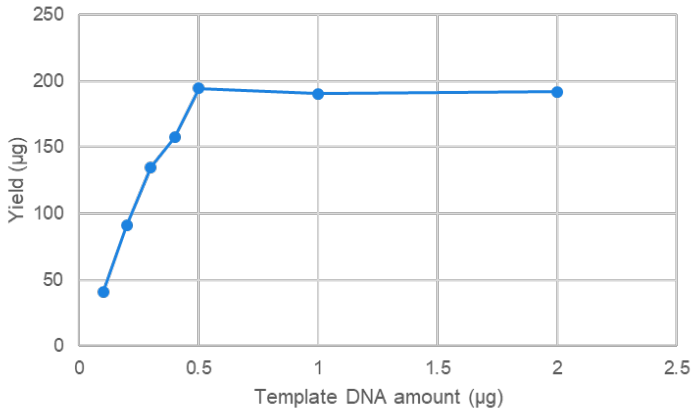

Fig. 5. Correlation between amount of template DNA and RNA yield.

Example 3-A

< Method >
IVT reactions were performed at various reaction volume (20 - 200 µl) with CleanCap Reagent AG (3′ OMe) using the Positive Control Template (FLuc). Details other than reaction volume are as described in this Manual.

< Results >
Total amount of RNA obtained was proportional to the reaction volume. However, the concentration and the yield per 20 µl reaction was not affected by scaling up the reaction volume.

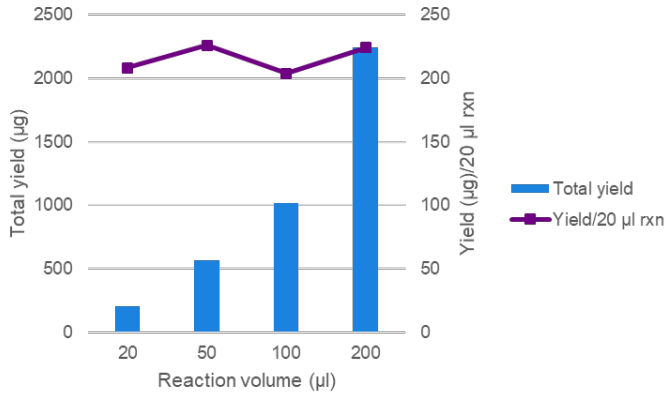

Fig. 6A. Correlation between IVT reaction volume and RNA yield.

Example 3-B

< Method >
1 ng of the RNA product obtained in Example 3-A was examined by Bioanalyzer.

< Results >
The IVT reaction volume did not affect the quality of the RNA product.

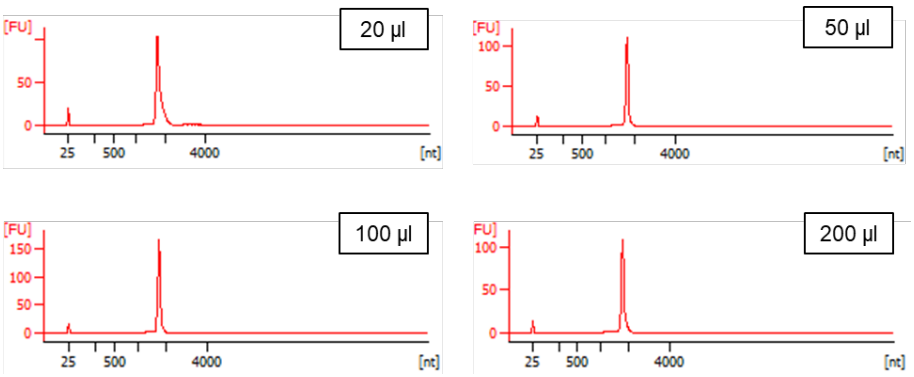

Fig. 6B. Analysis of the RNA product with Bioanalyzer.

Example 4-A

< Method >
IVT reactions were performed with various reaction times using the Positive Control Template (FLuc) with CleanCap Reagent AG (3′ OMe) and N^1-methyl pseudo UTP.

< Results >
In the case of 1.9 kb long synthesized mRNA, the RNA yield peaked in about 1 hour, after which no change was observed (for 1 to 16 hours).

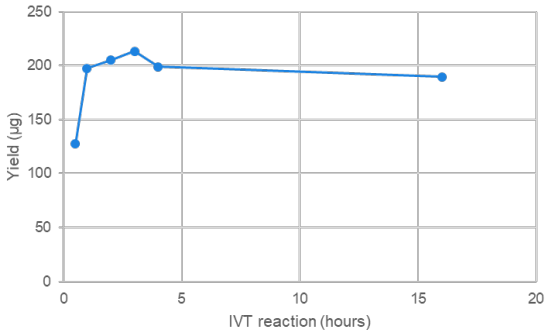

Fig. 7A. Correlation between IVT reaction time and RNA yield.

Example 4-B

< Method >
1 ng of the RNA product obtained in Example 4-A was analyzed by Bioanalyzer.

< Results >
No changes were observed for different reaction times.

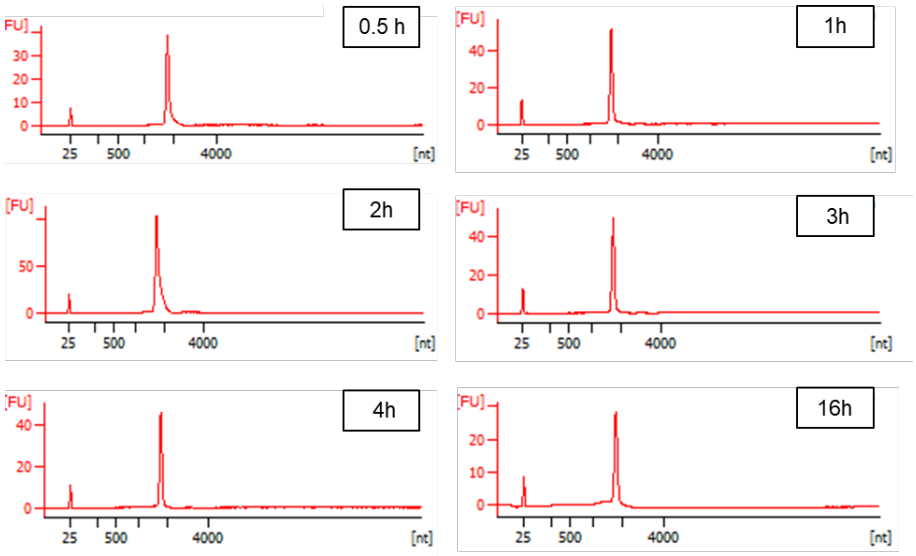

Fig. 7B. Comparison of RNA quality over reaction time using Bioanalyzer.

Example 5-A

< Method >
FLuc mRNA was synthesized in an IVT reaction with CleanCap Reagent AG (3′ OMe) and N^1-methyl pseudo UTP, and was purified by LiCl precipitation or spin column method using NucleoSpin kit.

< Results >
The amount of RNA obtained was less for one elution with the spin column method (Elution x1) compared to the LiCl precipitation method, but the amount of RNA with two elutions (Elution x2) with the spin column was approximately same as that with LiCl method. We strongly recommended performing elution twice when using the spin column method.

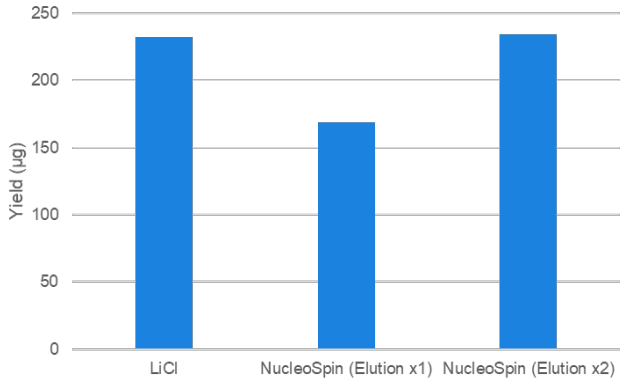

Fig. 8A. Comparison of RNA purification between LiCl precipitation and spin column methods.

Example 5-B

< Method >
HEK293T cells were transfected with 0.5 µg of the RNA obtained in Example 5-A, using the *Trans*IT-mRNA Transfection Kit.

< Results >
The FLuc activity in cells recovered after 24 hours was equivalent to that of the commercially available FLuc mRNA positive control.

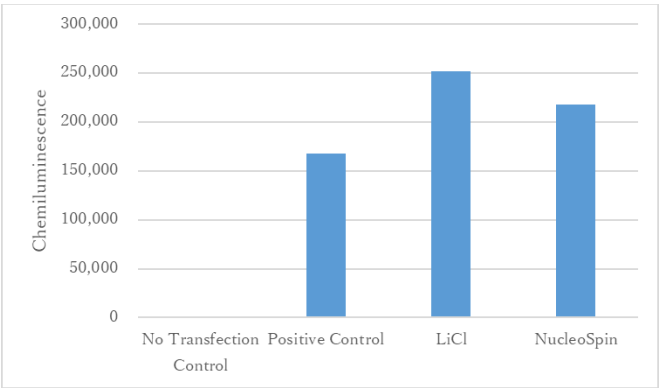

Fig. 8B. Expression of FLuc mRNA purified by different methods in HEK293T cells.

XII. Troubleshooting

Problems obtaining the desired IVT template plasmid

See the troubleshooting guide for In-Fusion cloning and "In-Fusion cloning tips and FAQs" in the user manual for In-Fusion Snap Assembly Master Mix (Cat. #638943/638944/638947 - 638949).

See our website: https://www.takarabio.com/in-fusion

For other problems

Problem	Cause	Solution
RNA yield is less	RNase contamination of template DNA	Perform phenol-chloroform extraction of template DNA after restriction digestion and then purify by ethanol precipitation.
	Amount of template DNA is too little	Check the amount of template DNA by agarose gel electrophoresis. If there is a discrepancy with the OD measured value, try purifying the template DNA again.
	Reaction time is insufficient	Lengthen the IVT reaction time.
	RNA length is less than 300 bases or RNA concentration is less than 0.1 μg/μl	Instead of purifying by the LiCl purification method, try using the spin column method or ethanol precipitation method after phenol-chloroform extraction.
	RNA pellet loss	Remove the supernatant carefully using a micropipette with as small a tip as possible.
	Insufficient RNA dissolution/elution	Dissolution may take time depending on the RNA yield. Allow the solution to stand at room temperature or 4℃, mix as needed, and then measure the RNA after it has completely dissolved. If it still does not dissolve, add some more solution. When the spin column method is used for purification, a single round of elution may not be sufficient. It is strongly recommended that you perform elution twice (e.g., 50 μl x 2).
	RNase contamination of reagent, equipment, or during processing	Take precautions against RNase contamination by using dedicated tubes and micropipette tips for the reaction and wearing new disposable gloves.
	Reagent deterioration	The enzymes should be stored at -20℃ and kept on ice. Avoid excessive agitation and freezing/thawing. If at least 100 μg of RNA cannot be obtained with (PCT) Positive Control Template (FLuc), it is time to repurchase.
The RNA obtained is larger than the desired size	Linearization of template plasmid is insufficient	Repeat restriction enzyme treatment of the template plasmid and confirm complete plasmid linearization with agarose gel electrophoresis before using the template.
	Denaturation of RNA is insufficient	Perform electrophoresis with denaturing agarose or acrylamide gel.
RNA that is smaller than the desired size	The CDS contains a sequence similar to the transcription termination signal of T7 RNA Polymerase	If possible, change the sequence. When changing the coding sequence, change the codons keeping the amino acid sequence the same.
Fragmented RNA smaller than the desired size is observed	RNase contamination	Take precautions against RNase contamination by using dedicated tubes and micropipette tips in the reaction and wearing new disposable gloves.

XIII. References

1) Karikó, K. *et al.* Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Mol Ther J Am Soc Gene Ther.* (2008) **16:** 1833-1840.
2) Vaidyanathan, S. *et al.* Uridine Depletion and Chemical Modification Increase Cas9 mRNA Activity and Reduce Immunogenicity without HPLC Purification. *Mol Ther Nucleic Acids.* (2018) **12:** 530-542.
3) Xia, X. Detailed Dissection and Critical Evaluation of the Pfizer/BioNTech and Moderna mRNA Vaccines. *Vaccines (Basel).* (2021) **9:** 734.
4) Schenborn, E. T. and Mierindorf, R. C. A novel transcription property of SP6 and T7 RNA polymerases: dependence on template structure. *Nucleic Acids Res.* (1985) **13:** 6223-6236.

XIV. Related Products

Cloning Kit for mRNA Template (Cat. #6143)
Takara IVTpro™ T7 mRNA Synthesis Kit (Cat. #6144)
PrimeSTAR® Max DNA Polymerase (Cat. #R045A/B)
TaKaRa Ex Premier™ DNA Polymerase (Cat. #RR370S/A/B, RR371S/A/B)
Stellar™ Competent Cells (Cat. #636763)
NucleoSpin Gel and PCR Clean-up (Cat. #740609.10/.50/.250)*
NucleoSpin Plasmid (Cat. #740588.10/.50/.250)*
*Hin*d III (Cat. #1060A/B)
NucleoSpin RNA Clean-up (Cat. #740948.10/.50/.250)*
In-Fusion Snap Assembly Master Mix (Cat. #638943/638944/638947 - 638949)

* Not available in all geographic locations. Check for availability in your area.